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Cellular Clocks: Coupled Circadian and Cell Division Cycles

Dispatch

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Gating of cell division by the circadian clock is well known, yet its mechanism is little understood. Genetically tractable model systems have led to new hypotheses and questions concerning the coupling of these two cellular cycles.

Most cells have regulatory pathways controlling replication and others that govern temporal organization. The cell cycle takes the cell through mitosis, and the circadian clock takes it through the day. In the former case, the time required to complete a cycle can range from minutes to days, varying with temperature, nutrition or other factors. In the latter case, a circa 24 hour period is always maintained, with great precision and over a wide range of conditions (circadian clocks are compensated for changes in the environment, such as nutrition or average temperature). While the cell cycle is characterized by discrete checkpoints and stages, the circadian program is a continuum, with reference points, or phases, designated almost arbitrarily, usually according to our concept of night and day. Two recent papers [1,2], one of which was recently published in *Current Biology*, explore the link between the cell cycle and the circadian clock using model systems.

Bi-cycles

The cell cycle and the circadian clock could theoretically coexist without taking any notice of each other. But the circadian clock is known to modulate many functions within cells, from gene expression to signaling strength. In each of these cases, quantitative changes occur systematically and predictably over 24 hours. As for circadian control of the cell cycle, the gating of cell division to a certain time of day is well described for organisms as diverse as unicells [3–5] and mammals [6]. Yeast does not even have an overt circadian rhythm, yet its cell division is modulated, in some conditions, every 24 hours. As for the inverse, i.e. the influence of the cell cycle on circadian timing, there is no evidence to support this idea as yet. For example, a resilient 24 hour rhythm in gene expression continues unchanged in cyanobacteria that replicate with a period of about 10 hours [4].

Both circadian and cell division cycles are described by powerful sets of genetic tools, and recent studies have used these tools together with model systems to study the coupling between the two timing systems. A first genetic link was established with the description of circadian regulation of cell cycle genes in the liver, detected using microarray analysis [7,8]. In one of the recent studies [2], mitotic cells have been shown to correlate with time-of-day rather than time-of-injury in regenerating mouse livers. This work also shows direct regulation of a cell cycle gene by circadian clock components, which for example activate an E-box in the *wee1* promoter. Thus, circadian gating of the cell cycle could, for example, be due to circadian expression of *wee1*.

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A Fish with a Bi-cycle

In a recent issue of *Current Biology*, Dekens *et al.* [1] describe a daily rhythm in the cell cycle of developing zebrafish larvae. At 25°C, the embryos hatch at around day 4. On day 6, a circadian regulation of S-phase nuclei becomes apparent if the larvae are exposed to a light–dark cycle (mimicking a 12 hour day). The rhythm continues, though with lower amplitude, after release to constant darkness. This indicates 'circadian' regulation, as opposed to a direct light response.

There are some features of the zebrafish which make it especially apt for studying the coupling of the cell cycle to the circadian clock. As with other vertebrates, multiple tissues and organs in the zebrafish have functioning cellular circadian clocks [9]. But, unlike many other animals, zebrafish are transparent, and apparently all of their cells are light sensitive. Thus, all cellular circadian clocks can be directly entrained without specialized light-harvesting organs, such as eyes. Coupled circadian and cell cycling was characterized in epidermal cells, heart, gut and even a fibroblast-like PAC-2 cell line [1], so any of these tissues might be adopted as an optimal experimental tool, depending on the question. Zebrafish are a model organism for vertebrate development, yet they are not homeotherms. Thus the coupling mechanism might show similarities both with organisms that regulate their temperature, such as mammals, and those that don't, such as unicells.

Re-cycle

Because the circadian clock regulates so many cellular processes, its control points in the cell cycle could occur at many stages. How might this be attacked experimentally? A straightforward, molecular approach might go through all known components of the cell cycle, looking for circadian regulation. For example, histone acetylation modulates clock gene expression and also regulates the cell cycle on several levels [10–12]. Thus, this could be one coupling pathway.

Alternatively, a conceptual approach, developing hypotheses and questions, might be useful. Here, we recycle a model for sleep behavior [13] and project it to the coupling of cell and circadian cycles (Figure 1). Like the cell cycle, sleep is regulated by the circadian system. It normally occurs every 24 h, but in special conditions and in some animals, it can occur more or less frequently. The 'two process' model predicts all of these sleep–wake patterns, and experiments can be designed to probe its application to the circadian cell cycle coupling phenomenon. The fundamental

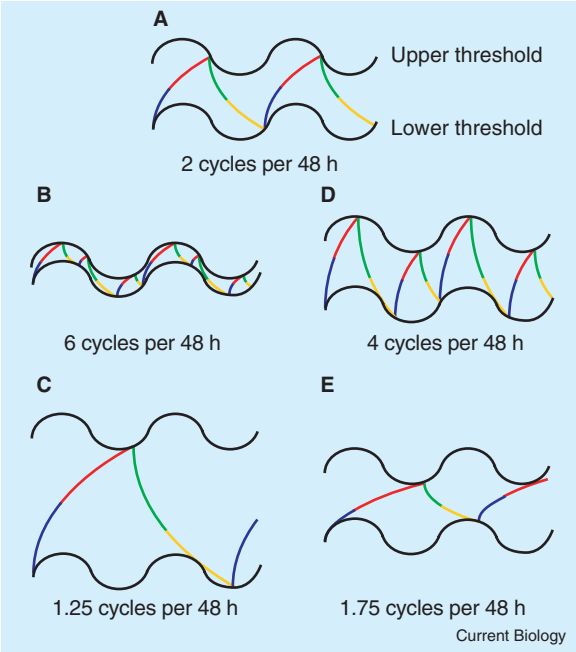

Figure 1. The 'two process' model for sleep [13] can be extended to cell cycle regulation.

Circadian oscillations continue with a 24 hour period (2 cycles or days are shown for each panel) over a variety of conditions, creating oscillating upper and lower thresholds for sleep propensity or the cell cycle (A). The second process is characterized by stages, and the time it takes to complete a stage can be modified by changing thresholds (A versus B or C) or by changing rates (A versus D or E). Even in the absence of obvious circa 24 hour gating, the circadian clock can be modulating the second process, namely, sleep or the cell cycle. The stages of the cell cycle are arbitrarily designated here by blue, red, green and yellow, for G1, S, G2 and M, respectively.

components of the model include a stable, 24 hour oscillation and a second, downstream process — sleepiness and wakefulness, as published, or in the case at hand, the different stages of the cell cycle. For the sleep–wake cycle, checkpoints are when we fall asleep and when we wake up. The thresholds determining these checkpoints vary with time of day, as we are more or less susceptible to fall asleep (upper threshold) or ready to wake up (lower threshold). As for the cell cycle, any or all stages or checkpoints could be used, and could vary according to organism.

How does the model account for changing, non-24 hour periods in the downstream process (sleep or cell cycle)? Both the lower and the upper threshold change predictably due to the precision of the circadian clock. In Figure 1A, the second process hits each of the respective thresholds every 24 hour. If, however, the height between the thresholds is changed (Figure 1B,C), then faster, slower or even quite irregular cycling occurs. Different cycle lengths could also be achieved if the rate of progression through the checkpoints changes while the distance between upper and lower thresholds remains the same (Figure 1D,E). Thus, robust circadian gating of the cell cycle occurs only under special conditions when the two timing processes acquire resonance.

A fundamental assumption of the model is the circadian regulation of thresholds for progression from one checkpoint to the next. Could thresholds for cell cycle progression vary over the day (remember that many cellular processes are controlled by the clock)? In mouse liver, expression of both *wee1* and its target, *cdc2*, show circadian rhythms, but not exactly in phase with each other [2]. WEE1 regulates the cell cycle through CDC2. Thus, the concentration of *wee1* required for regulation at a given time of day — the threshold amount — will vary according to the (circadianly regulated) amount of its target *cdc2*.

Here, we promote combining wet (e.g., fish) and dry (e.g., modeling) experiments to characterize coupling of the circadian clock and the cell cycle. Understanding the coupling of these extensive cellular genetic networks should be of interest to many biologists, including those who study normal development, cancer growth, and cellular immunology.

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